British Journal of Haematology, 2003, **122**, 471–478

Prothrombotic coagulation defects and cardiovascular risk factors in young women with acute myocardial infarction

BEA C. TANIS,[1] DAISY G. M. BLOEMENKAMP,[2] MAURICE A. A. J. VAN DEN BOSCH,[2] JEANET M. KEMMEREN,[2] ALE ALGRA,[2,3] YOLANDA VAN DE GRAAF[2] AND FRITS R. ROSENDAAL[1,4] [1]*Thrombosis and Haemostasis Research Centre, Department of Haematology, Leiden University Medical Centre,* [2]*Julius Centre for General Practice and Patient Oriented Research,* [3]*Department of Neurology, University Medical Centre Utrecht, and* [4]*Department of Clinical Epidemiology, Leiden University Medical Centre, the Netherlands*

Received 9 August 2002; accepted for publication 9 April 2003

Summary. We investigated the effect of prothrombotic coagulation defects in combination with smoking and other conventional risk factors on the risk of myocardial infarction in young women. In 217 women with a first myocardial infarction before the age of 50 years and 763 healthy control women from a population-based case–control study, factor V Leiden and prothrombin 20210A status were determined. Data on major cardiovascular risk factors and oral contraceptive use were combined with the presence or absence of these prothrombotic mutations, and compared between patients and controls. The overall odds ratio for myocardial infarction in the presence of a coagulation defect was 1·1 [95% confidence interval (CI) 0·6–1·9]. The combination of a prothrombotic mutation and current smoking increased the risk of myocardial infarction 12-fold (95% CI 5·7–27) compared with non-smokers without a coagulation defect. Among women who smoked cigarettes, factor V Leiden presence versus absence increased the risk of myocardial infarction by 2·0 (95% CI 0·9–4·6), and prothrombin 20210A presence versus absence had an odds ratio of 1·0 (95% CI 0·3–3·5). We conclude that factor V Leiden and prothrombin 20210A do not add substantially to the overall risk of myocardial infarction in young women. However, in women who smoke, the presence of factor V Leiden increased the risk of myocardial infarction twofold.

Keywords: myocardial infarction, factor V Leiden, prothrombin 20210A mutation, risk factors, oral contraceptives.

Factor V Leiden and the prothrombin 20210A variant are prothrombotic mutations that predispose for venous thrombosis (Bertina *et al*, 1994; Poort *et al*, 1996; Bloemenkamp *et al*, 1995); however, their contribution to the risk of arterial thrombosis is less clear. Positive associations were found in specific groups of patients, especially young patients with other cardiovascular risk factors or patients with normal coronary angiographies (Rosendaal *et al*, 1997a, b; Inbal *et al*, 1999; Mansourati *et al*, 2000; Van de Water *et al*, 2000; Burzotta *et al*, 2002), but these could not be confirmed in several other studies (Emmerich *et al*, 1995; Prohaska *et al*, 1995; Ridker *et al*, 1995a, 1999; Van Bockxmeer *et al*, 1995; Ardissino *et al*, 1996; Corral *et al*, 1997; Amowitz *et al*, 1999; Croft *et al*, 1999; Irani-Hakime *et al*, 2001; Smiles *et al*, 2002). In a study of 84 women with myocardial infarction aged below 44 years of age, factor V Leiden increased the risk of myocardial infarction 2·4-fold, an effect which seemed to be confined to current smokers (Rosendaal *et al*, 1997b). A similar effect of other major cardiovascular risk factors was seen for carriers of prothrombin 20210A (Rosendaal *et al*, 1997a).

Factor V Leiden enhances the effect of oral contraceptives, with a synergistic risk of venous thrombosis 35-fold greater than that of non-users without factor V Leiden (Vandenbroucke *et al*, 1994). Moreover, the increased risk of thrombosis is most pronounced in the first year of oral contraceptive use, especially in women with thrombophilic defects (Herings *et al*, 1999; Bloemenkamp *et al*, 2000; Vandenbroucke *et al*, 2000). For the prothrombin 20210A mutation and oral contraceptive use, a synergistic risk has been found for deep vein thrombosis and cerebral venous sinus thrombosis (Martinelli *et al*, 1998; 1999).

Oral contraceptives increase the risk of myocardial infarction with estimated relative risks between 2 and 5, which may be reduced by abstinence from smoking and a

Correspondence: Prof Dr F. R. Rosendaal, Department of Clinical Epidemiology and Department of Haematology, Leiden University Medical Centre, Building 1, C9, PO Box 9600, 2300 RC Leiden, the Netherlands. E-mail: F.R.Rosendaal@lumc.nl

blood pressure check prior to prescription of oral contraceptives (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1997). We recently found that modern low-dose oral contraceptives still increase the risk of myocardial infarction twofold and that the risk associated with oral contraceptive use was similar in women with and without a prothrombotic mutation (Tanis *et al*, 2001).

In this nationwide population-based case–control study we examined the effect of factor V Leiden and prothrombin 20210A on the first myocardial infarction in women aged less than 50 years. Furthermore, we aimed to assess whether these mutations would help to identify women at a high risk when smoking cigarettes, with a history of hypertension, diabetes or hypercholesterolaemia, and during oral contraceptive use.

MATERIALS AND METHODS

Design. The Risk of Arterial Thrombosis In relation with Oral contraceptives (RATIO) study is a population-based case–control study on the association between oral contraceptive use and acute myocardial infarction, performed in the Netherlands (Tanis *et al*, 2001). We included patients from all eight university hospitals as well as eight surrounding hospitals and a population-based control group matched for age, index year and area of residence. In the first phase of the study a standardized mailed questionnaire was completed by 248 patients and 925 controls, and in the second phase either blood samples were drawn or buccal swabs collected for the determination of factor V Leiden and prothrombin 20210A status. The Ethics Committees of all participating hospitals approved the study protocol.

Patients and control women. Patients were women aged between 18 and 49 years with a first myocardial infarction who were all hospitalized in university or general hospitals in the Netherlands in the period between January 1990 and October 1995 (see Appendix for participating centres). Medical records and discharge letters were reviewed for confirmation of the diagnostic criteria for myocardial infarction, which was defined by the presence of symptoms, diagnostic elevated cardiac enzymes, and electrocardiographic changes indicative of myocardial infarction (Fried *et al*, 1991). We obtained DNA from 204 patients by venous blood draw and from 13 patients by mailed-in buccal swabs. Control subjects were women aged between 18 and 49 years without a prior history of myocardial infarction, stroke or severe peripheral arterial disease, and recruited from the same areas using random digit dialling (Hartge *et al*, 1984). In the control group, DNA was obtained from 763 women (638 women participated in venous blood draw and from 131 women we obtained a buccal swab). Overall response was 75%.

Demographic characteristics. Data regarding cigarette smoking, a history of hypertension, diabetes and hypercholesterolaemia, height and weight, educational level, a family history of cardiovascular disease and oral contraceptive use were collected with standardized mail questionnaires in 1997 and 1998. All questions elicited information from a time period preceding the index date, i.e. the date of myocardial infarction in patients and the mid-year of the same year in controls. Current smokers were those who reported smoking regularly in the year before the index date. Women were classified as having a history of hypertension, diabetes or hypercholesterolaemia when they reported a physician's diagnosis or were taking medication for these conditions before the index date. At the time of venous blood draw, we were able to confirm the data on three metabolic risk factors: hypertension, diabetes and hypercholesterolaemia. A women was classified as hypertensive when using antihypertensive medication, having a systolic blood pressure of 160 mmHg or higher, or a diastolic blood pressure of 95 mmHg or higher at the time of venepuncture. Blood pressure was measured in a supine position after 5 min rest. Diabetes was defined as the use of glucose lowering medication or a non-fasting glucose above 11·0 mmol/l. Hypercholesterolaemia was defined as the use of medication for this condition or a non-fasting serum total cholesterol above 6·5 mmol/l. Body mass index was calculated as body weight (kg) divided by height squared (m^2). Women who had a body mass index $\geq 27{\cdot}3$ kg/m^2 were classified as obese to facilitate comparisons with earlier reports (Fried *et al*, 1991; Rosendaal *et al*, 1997a). A family history of cardiovascular disease was defined as the presence of myocardial infarction, stroke or peripheral vascular disease under 60 years of age in a first-degree relative. Women were classified as current oral contraceptive users if they had used oral contraceptives in the month before the index date.

Blood collection and laboratory analysis. Venepunctures and buccal swab collections were performed between June 1998 and May 2000. Blood samples were drawn from the antecubital vein into two Sarstedt Monovette® tubes containing 0·106 mol/l trisodium citrate and centrifuged for 20 min at 4500 r.p.m. High-molecular-weight DNA was isolated from the white blood cells by a salting-out process (Miller *et al*, 1988) or from buccal swabs (Thomson *et al*, 1992). Cotton swabs in sodium dodecyl sulphate (SDS)-proteinase K solution were incubated for 2 h at 65°C after arrival and centrifuged for 1 min at 1000 r.p.m. Potassium acetate was added to a final concentration of 1·6 mol/l and after 15 min incubation on ice, protein were removed using chloroform/isoamylalcohol (24 : 1) treatment. The DNA was stored at 4°C until amplification. Genetic analysis of the factor V Leiden mutation (1691 G → A) was performed with the polymerase chain reaction as we described previously (Bertina *et al*, 1994). The prothrombin variant (20210 G → A) was determined by the presence of the HindIII restriction site in the polymerase chain reaction fragment also described previously (Poort *et al*, 1996). The technician who performed DNA analyses was blinded to whether a blood sample was from a patient or a control.

Statistical analysis. The prevalence of factor V Leiden and prothrombin 20210A was calculated in patients and controls separately. Univariate odds ratios with 95% confidence intervals (95% CI) were calculated for the relationship between coagulation defects or other cardiovascular risk factors and myocardial infarction as a measure of

relative risk The effect of a combination of risk factors i e coagulation defects and oral contraceptive use or coagulation defects and major cardiovascular risk factors were analysed by calculating odds ratios in subjects with either one or both of these risk factors, compared with those with neither risk factor Adjustment when appropriate, was performed by multivariate regression models that controlled for age, index year and area of residence

RESULTS

Risk factor variables

The mean age of the 217 patients with a first myocardial infarction was 42 8 years, and that of 763 control women 38 7 years (Table I) As expected, patients more frequently had a history of hypertension (25% *vs* 6%) diabetes (5% *vs* 1%) or hypercholesterolaemia (11% *vs* 3%) Validation of the metabolic risk factors at the time of blood sampling confirmed the diagnoses of hypertension (82% *vs* 12%), diabetes (11% *vs* 2%) or hypercholesterolaemia (62% *vs* 16%) However, as the result of blood pressure-lowering drugs as well as lipid-lowering drugs, prescribed in patients after myocardial infarction, the percentages for hypertension and hyperlipidaemia far exceeded those documented prior to myocardial infarction (physician's diagnosis or drug therapy) The most striking difference between patients and controls was the percentage smoking at the index date 83% in patients versus 42% in controls

Prothrombotic mutations

The factor V Leiden mutation was found in 13/217 patients (6 0%), and in 42/763 healthy control women (5 5%) The prothombin 20210A mutation was present in 5/217 (2 3%) patients and 18/763 control women (2 3%) This yielded an overall odds ratio of 1 1 (95% CI 0 6–2 1) for first myocardial infarction in the presence of factor V Leiden and 1 0 (95% CI 0 4–2 7) for the prothrombin mutation (Table II) There were no homozygous carriers of either factor V Leiden or prothrombin 20210A two control women were heterozygous for both mutations Eighteen patients (8 3%) and 58 controls (7 6%) were carriers of either factor V Leiden or prothrombin 20210A Adjustment for the stratification variables (age index year and area of residence) did not affect the estimates In women with a prothrombotic mutation aged less than 35 years, the relative risk of myocardial infarction was 1 6 (95% CI 0 4–5 8) and in women older than 35 years it was 0 9 (95% CI 0 5–1 7) The difference in these odds ratios was completely determined by factor V Leiden (and not by prothrombin 20210A) with a relative risk of 2 3 (95% CI 0 6–8 6) for women less than 35 years and 0 9 (95% CI 0 4–1 9) for women older than 35 years

Combined effect of prothrombotic mutations and cardiovascular risk factors

The risk of myocardial infarction was increased more than 12-fold among smoking women with a coagulation defect compared with non-smokers without a coagulation defect indicating a synergistic effect of both risk factors The adjusted odds ratio for the combination of hypertension and a coagulation defect was 11 1 (95% CI 1 1–114) although this was based on very small numbers The results presented in Table III indicate that the combined effect of a coagulation defect and either hypercholesterolaemia or obesity did not exceed the separate effect of the two factors For diabetes either the small sample size or an absence of carriers of a coagulation defect did not allow analyses for interaction When we combined the presence of one or more of the metabolic risk factors (hypertension, hypercholesterolaemia diabetes and obesity) the odds ratio did not differ between women without (OR 3 3 95% CI 2 4–4 6) or with (OR 3 3 95% CI 1 4–7 7) a coagulation defect The risk of myocardial infarction was increased twofold for oral

Table I Baseline characteristics of women with first myocardial infarction and control women

	Patients (n = 217) N (%)	Control women (n = 763) N (%)
Age (year)		
Mean (SD)	42 8 (6 1)	38 7 (8 0)
Range	24–49	18–49
Caucasian ethnicity*	206 (95)	719 (95)
Oral contraceptive use*	85 (39)	276 (36)
Education level*		
Primary school or less	113 (52)	229 (30)
Secondary school	80 (37)	332 (44)
Higher education or university	24 (11)	198 (26)
History of		
Hypertension	55 (25)	47 (6)
Hypercholesterolaemia*	23 (11)	22 (3)
Diabetes	11 (5)	10 (1)
Currently treated for		
Hypertension†	177 (82)	92 (12)
Hypercholesterolaemia†	134 (62)	125 (16)
Diabetes†	23 (11)	12 (2)
Body Mass Index (mean SD)*	25 5 (5 0)	23 4 (3 7)
Cigarette smoking		
Never	19 (9)	248 (32)
Former	18 (8)	198 (26)
Current	181 (83)	319 (42)
Family history of cardiovascular disease*	141 (67)	262 (37)

Data are numbers (%) unless otherwise indicated N number of patients SD standard deviation

* Data was missing on the ethnicity of four control women oral contraceptive use of eight control women and three patients education level of four control women hypercholesterolaemia of five control women cigarette smoking of four control women family history of cardiovascular disease of six patients and 48 control women

† Taking prescription drugs for these conditions or validated by a cholesterol level > 6 5 mmol/l non fasting serum glucose > 11 1 mmol/l systolic blood pressure > 160 mmHg or diastolic blood pressure > 95 mmHg

Table II. Prevalence of factor V Leiden and 20210A mutation in the prothrombin gene in 217 women with first myocardial infarction and 763 healthy control women

	Patients N (%)	Control women N (%)	Odds ratio (95% CI)
Factor V Leiden*			
1691 GG	204 (94 0)	721 (94 5)	1 0†
1691 AG	13 (6 0)	42 (5 5)	1 1 (0 6–2 1)
Prothrombin*			
20210 GG	212 (97 7)	745 (97 6)	1 0†
20210 AG	5 (2 3)	18 (2 4)	1 0 (0 4–2 7)
Coagulation defect			
1691 GG or 20210 GG	199 (91 7)	705 (92 4)	1 0†
1691 AG or 20210 AG	18 (8 3)	58 (7 6)‡	1 1 (0 6–1 9)

N, number of patients
*GG indicates wild-type genotype AG indicates heterozygosity for the mutation
†Reference category
‡Two control women were heterozygous for both factor V Leiden and prothrombin 20210A

contraceptive users without a prothrombotic mutation (OR 2 1, 95% CI 1 5–3 0) and was not more increased in those with a prothrombotic mutation (OR 1 9, 95% CI 0 6–5 5)

Smokers

Table IV shows that, in the subgroup of smokers, the presence of factor V Leiden increased the risk of myocardial infarction by 2 0 (95% CI 0 9–4 6), with an adjusted odds ratio of 3 4 (95% CI 1 3–9 2) In carriers of prothrombin 20210A who smoked, no additional increase in risk was found, with an odds ratio of 1 0 (95% CI 0 3–3 5) (adjusted OR 1 1, 95% CI 0 3–4 7) The combined effect of smoking, oral contraceptive use and a coagulation defect had a high, adjusted odds ratio of 34 (95% CI 5 5–179) compared with women without any of these risk factors

Combination of cardiovascular risk factors

The contribution of conventional risk factors (smoking, hypertension, diabetes, hypercholesterolaemia and obesity) was far more than that of factor V Leiden, and in only four patients (2%) none of these risk factors were found, compared with 128 controls (18%) The risk of myocardial infarction increased very sharply with the number of risk factors observed those with one risk factor had a

Table III. Effect of smoking, hypertension, hypercholesterolaemia diabetes obesity and oral contraceptive use with and without a coagulation defect on the risk of first myocardial infarction

Cardiovascular risk factor	Presence of coagulation defect	Patients N (%)	Control women N (%)	Odds ratio (95% CI)
No smoking	No coagulation defect	35 (16 1)	403 (52 1)	1 0*
	FVL/20210A	2 (0 9)	38 (5 0)	0 5 (0 1–2 3)
Smoking	No coagulation defect	164 (75 6)	300 (39 5)	6 5 (4 3–9 7)
	FVL/20210A	16 (7 4)	18 (2 4)	12 5 (5 7–27)
Non-hypertensive	No coagulation defect	147 (67 7)	657 (86 6)	1 0*
	FVL/20210A	15 (6 9)	55 (7 2)	1 2 (0 7–2 2)
Hypertensive	No coagulation defect	52 (24 0)	46 (6 1)	4 2 (2 7–6 6)
	FVL/20210A	3 (1 4)	1 (0 1)	11 1 (1 1–114)
Non-hypercholesterolaemic	No coagulation defect	178 (82 0)	682 (90 0)	1*
	FVL/20210A	16 (7 4)	54 (7 1)	1 2 (0 6–2 1)
Hypercholesterolaemic	No coagulation defect	21 (9 7)	19 (2 5)	3 7 (1 9–7 3)
	FVL/20210A	2 (0 9)	3 (0 4)	1 7 (0 3–10 4)
Non-diabetic	No coagulation defect	188 (86 6)	693 (91 2)	1*
	FVL/20210A	18 (8 3)	57 (7 5)	1 2 (0 6–2 0)
Diabetic	No coagulation defect	11 (5 1)	10 (1 3)	4 0 (1 6–9 8)
	FVL/20210A	–	–	–
Non-obese	No coagulation defect	143 (65 9)	604 (81 5)	1*
	FVL/20210A	10 (4 6)	47 (6 3)	0 9 (0 4–1 9)
Obese	No coagulation defect	56 (25 8)	82 (11 1)	2 6 (1 7–3 8)
	FVL/20210A	8 (3 7)	8 (1 1)	3 7 (1 3–10 2)
No oral contraceptive use	No coagulation defect	116 (54 2)	443 (58 7)	1*
	FVL/20210A	13 (6 1)	36 (4 8)	1 4 (0 7–2 7)
Oral contraceptive use	No coagulation defect	80 (37 4)	256 (33 9)	2 1 (1 5–3 0)
	FVL/20210A	5 (2 3)	20 (2 6)	1 9 (0 6–5 5)

N number of patients FVL factor V Leiden Odds ratios adjusted for the stratification variables (age area of residence and calendar year)
*Reference category

Table IV. The effect of factor V Leiden and prothrombin 20210A mutation in smokers with and without myocardial infarction

	Patients N (%)	Control women N (%)	Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
Factor V Leiden				
1691 GG	168 (92 3)	307 (96 5)	1†	1†
1691 AG	12 (6 7)	11 (3 5)	2 0 (0 9–4 6)	3 4 (1 3–9 2)
Prothrombin				
20210 GG	176 (97 8)	311 (97 8)	1†	1†
20210 AG	4 (2 2)	7 (2 2)	1 0 (0 3–3 5)	1 1 (0 3–4 7)
Coagulation defect				
1691 GG or 20210 GG	164 (91 1)	300 (94 3)	1†	1†
1691 AG or 20210 AG	16 (8 9)	18 (5 7)	1 6 (0 8–3 3)	2 5 (1 1–5 6)

N, number of patients
*Odds ratios adjusted for the stratification variables (age area of residence and calendar year)
†Reference category
GG indicates wild-type genotype AG indicates heterozygosity for the mutation AA (homozygosity for the mutation) was not found

2 8-fold increased risk, those with two risk factors had a 14-fold increased risk, those with three risk factors a 26-fold increased risk, those with four risk factors a 89-fold increased risk and those with five risk factors had an odds ratio exceeding 140, all relative to those with no risk factor at all

DISCUSSION

In this large population-based case–control study the prevalence of factor V Leiden and prothrombin 20210A did not differ between patients and control women Factor V Leiden did not exert an effect on the overall risk of myocardial infarction (OR 1 1, 95% CI 0 6–2 1), nor did prothrombin 20210A (OR 1 0, 95% CI 0 4–2 7) The risk of myocardial infarction was further increased between twofold and threefold by factor V Leiden among smokers, but not by prothrombin 20210A Among very young women, defined as aged below 35 years, factor V Leiden increased the risk of myocardial infarction twofold Users of low-dose oral contraceptives had a twofold higher risk than non-users (95% CI 1 5–2 8), which was not affected by the presence of a prothrombotic mutation (Tanis *et al*, 2001) The combination of a coagulation defect, smoking of cigarettes and oral contraceptive use increased the risk of myocardial infarction 34-fold

Combined effect of prothrombotic mutations and cardiovascular risk factors

The influence of coagulation defects on myocardial infarction has been investigated since 1995 with varying outcomes (Emmerich *et al*, 1995, Kontula *et al*, 1995, Ridker *et al*, 1995a, b, 1999, Van Bockxmeer *et al* 1995, Ardissino *et al*, 1996, Arruda *et al*, 1997 Ferraresi *et al*, 1997, Rosendaal *et al*, 1997a, b, Doggen *et al*, 1998 Eikelboom *et al*, 1998, Franco *et al*, 1998 Croft *et al*, 1999 Gardemann *et al*, 1999, Inbal *et al*, 1999, Coulet *et al*, 2000) Different results on the contribution of the coagulation defects seem to depend on the type of population which was studied, with positive associations in specific subgroups of young women (Holm *et al*, 1994, Rosendaal *et al*, 1997a, b), smokers and patients with metabolic risk factors (Doggen *et al*, 1998, Inbal *et al*, 1999) or patients with normal coronary angiographies (Mansourati *et al*, 2000)

Previously, we reported that factor V Leiden was a determinant of myocardial infarction in young American women, increasing the risk 2 5-fold and fourfold when other major risk factors for myocardial infarction were taken into account (Rosendaal *et al*, 1997b) For the 20210A mutation, an age-adjusted odds ratio of 4 0 (95% CI 1 1–15 1) for myocardial infarction was found (Rosendaal *et al*, 1997a) Both in the two studies by Rosendaal and colleagues and the present study, the prevalence of smoking in young women with myocardial infarction was very high, 74% and 83% respectively In the present study, we confirmed the hypothesis that smoking is a prerequisite for the effect of factor V Leiden in arterial thrombosis, but found no effect for prothrombin 20210A Possible explanations for the different conclusions concerning metabolic risk factors in the two studies are admixture of different cardiovascular risk factors, i e obesity, in a heterogeneous American population or variations in geographical distribution of the prothrombotic mutations between young women from the USA and the Netherlands (Ridker *et al*, 1997, Rosendaal *et al*, 1998)

Strengths and limitations of the study

The present study is one of the largest case–control studies among young women that has examined the association between genetic coagulation defects and the risk of myocardial infarction Both for patients and control women the response was high, at 80% and 73% respectively The high percentage of oral contraceptive use in our study, almost 40% in both patient and control group, also allowed us to study the combined effect of oral contraceptive use and two prothrombotic mutations on myocardial infarction In contrast with the risk of venous thrombosis we found no interaction between oral contraceptive use and these prothrombotic mutations with regard to the risk of myocardial infarction

The RATIO study is limited by the fact that we only studied women who survived myocardial infarction It is unlikely that coagulation defects play an important role in survival of acute myocardial infarction which is high in this age group (Dunn *et al* 2000) However the possibility that the prothrombotic mutations were associated with more severe myocardial infarction and acute death cannot be excluded In this case we would have underestimated the effect of prothrombotic mutations on the risk of myocardial infarction

Clinical implications

Myocardial infarction is a multicausal disease and several risk factors are needed before acute myocardial infarction occurs in young patients (Sidney *et al* 1996 Siscovick *et al* 1997) From a clinical perspective the results of our study may have important implications In the absence of an increased overall risk of myocardial infarction in carriers of the factor V Leiden mutation or prothrombin 20210A the benefit of screening young individuals for these coagulation defects seems absent In contrast our study underlines the importance of screening for high blood pressure diabetes and hypercholesterolaemia before prescribing the pill Firstly because of interaction between these conventional risk factors and oral contraceptive use as described in the first phase of the RATIO study (Tanis *et al* 2001) and secondly the absolute risk associated with oral contraceptive use increases exponentially because of the steeply rising incidence of myocardial infarction with age (Farley *et al* 1998) Smoking was by far the most striking risk factor for myocardial infarction in young women present in over 80% of the patients compared with 42% of control women Therefore the most important message for young women starting to use oral contraceptives is to have their blood pressure measured and to stop smoking Given the high percentage of smokers among young women this is the most important issue for the prevention of premature atherothrombotic events

ACKNOWLEDGMENTS

This study was supported by a grant from the Netherland Heart Foundation (grant 97 063) The authors thank the cardiologists of the participating centres (see *Appendix*) We thank Mrs M de Boer and Mrs E van Lunteren in assisting random digit dialling and venepunctures and Mrs A van Dam and Mrs J van Dam for administrative support We are indebted to Mrs C Krommenhoek-van Es who performed the DNA analyses and Dr H Vos for advice Finally we particularly thank all the women who participated in the RATIO project

APPENDIX PARTICIPATING CENTRES

Sint Antonius Hospital Nieuwegein Prof Dr N M van Hemel
University Medical Center Amsterdam Dr R J G Peters
Leiden University Medical Center Dr V Manger Cats
Rijnstate Hospital Arnhem Dr H A Bosker
Medical Center Haaglanden Westeinde Hospital The Hague Dr J Kolf
University Medical Centrum Nijmegen- Sint Radboud Prof Dr F W A Verheugt
Leyenburg Hospital The Hague Dr B J M Delemarre
University Medical Center Rotterdam-Dijkzigt Dr F A M Jonkman
University Medical Center Maastricht Dr F Vermeer
Rijnland Hospital Leiderdorp Dr C van Rees
Free University Medical Center Amsterdam Dr O Kamp
University Medical Center Utrecht Prof Dr E O Robles de Medina*
University Medical Center Groningen Dr M van den Berg
Bronovo Hospital The Hague Dr P R M van Dijkman
Sint Franciscus Gasthuis Rotterdam Dr A Schelling
Diaconessenhuis Leiden Dr S A G J Witteveen
*Deceased

REFERENCES

Amowitz L L Komaroff A L Miletich J P & Ridker P M (1999) Factor V Leiden is not a risk factor for myocardial infarction among young women *Blood* **93** 1432–1433

Ardissino D Peyvandi F Merlini P A Colombi E & Mannucci P M (1996) Factor V (Arg 506 –> Gln) mutation in young survivors of myocardial infarction *Thrombosis and Haemostasis* **75** 701–702

Arruda V R Annichino Bizzacchi J M Goncalves M S & Costa F F (1997) Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease *Thrombosis and Haemostasis* **78** 1430–1433

Bertina R M Koeleman B P Koster T Rosendaal F R Dirven R J De Ronde H van der Velden P A & Reitsma P H (1994) Mutation in blood coagulation factor V associated with resistance to activated protein C *Nature* **369** 64–67

Bloemenkamp K W Rosendaal F R Helmerhorst F M Buller H R & Vandenbroucke J P (1995) Enhancement by factor V Leiden mutation of risk of deep vein thrombosis associated with oral contraceptives containing a third generation progestagen *Lancet* **346** 1593–1596

Bloemenkamp K W Rosendaal F R Helmerhorst F M & Van denbroucke J P (2000) Higher risk of venous thrombosis during early use of oral contraceptives in women with inherited clotting defects *Archives of Internal Medicine* **160** 49–52

Burzotta F De Paciaroni K S V Chiusolo P Manzoli A Caso relli I Leone A M Rossi E Leone G Maseri A & Andreotti F (2002) Increased prevalence of the G20210A prothrombin gene variant in acute coronary syndromes without metabolic or acquired risk factors or with limited extent of disease *European Heart Journal* **23** 26 30

Corral J Gonzalez Conejero R Lozano M L Rivera J Heras I & Vicente V (1997) The venous thrombosis risk factor 20210A allele of the prothrombin gene is not a major risk factor for arterial thrombotic disease *British Journal of Haematology* **99** 304 307

Coulet F Godard V Verdy E & Soubrier F (2000) Lack of association of the prothrombin gene variant G20210A with myocardial infarction in Caucasian males *Thrombosis and Haemostasis* **83** 796 797

Croft S A Daly M E Steeds R P Channer K S Samani N J & Hampton K K (1999) The prothrombin 20210A allele and its

association with myocardial infarction *Thrombosis and Haemostasis*, **81**, 861–864

Doggen, C J , Cats, V M , Bertina, R M. & Rosendaal, F R (1998) Interaction of coagulation defects and cardiovascular risk factors increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A *Circulation*, **97**, 1037–1041

Dunn, N R , Arscott, A , Thorogood, M , Faragher, B , de Caestecker, L , MacDonald, T M., McCollum, C , Thomas, S & Mann, R D (2000) Regional variation in incidence and case fatality of myocardial infarction among young women in England, Scotland and Wales *Journal of Epidemiology and Community Health*, **54**, 293–298

Eikelboom, J W , Baker, R I., Parsons, R , Taylor, R R. & van Bockxmeer, F M (1998) No association between the 20210 G/A prothrombin gene mutation and premature coronary artery disease. *Thrombosis and Haemostasis*, **80**, 878–880

Emmerich, J , Poirier, O , Evans, A , Marques-Vidal, P , Arveiler, D., Luc, G , Aiach, M & Cambien, F (1995) Myocardial infarction, Arg 506 to Gln factor V mutation, and activated protein C resistance *Lancet*, **345**, 321

Farley, T M , Collins, J & Schlesselman, J J (1998) Hormonal contraception and risk of cardiovascular disease. An international perspective *Contraception*, **57**, 211–230

Ferraresi, P , Marchetti, G , Legnani, C , Cavallari, E , Castoldi, E , Mascoli, F , Ardissino, D , Palareti, G & Bernardi, F (1997) The heterozygous 20210 G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increased in frequency in artery disease *Arteriosclerosis Thrombosis and Vascular Biology*, **17**, 2418–2422

Franco, R F , Maffei, F H , Lourenco, D , Morelli, V , Thomazini, I A , Piccinato, C.E , Tavella, M H. & Zago, M A (1998) Factor V Arg306 -> Thr (factor V Cambridge) and factor V Arg306 -> Gly mutations in venous thrombotic disease *British Journal of Haematology*, **103**, 888–890

Fried, L P , Borhani, N O , Enright, P , Furberg, C D , Gardin, J M , Kronmal, R A , Kuller, L H , Manolio, T A , Mittelmark, M B & Newman, A (1991) The Cardiovascular Health Study design and rationale *Annals of Epidemiology* **1**, 263–276

Gardemann, A , Arsic, T , Katz, N , Tillmanns, H Hehrlein, F W & Haberbosch, W (1999) The factor II G20210A and factor V G1691A gene transitions and coronary heart disease *Thrombosis and Haemostasis*, **81**, 208–213

Hartge, P , Brinton, L A , Rosenthal, J F , Cahill, J I Hoover, R N & Waksberg, J (1984) Random digit dialing in selecting a population-based control group *American Journal of Epidemiology* **120**, 825–833

Herings, R M , Urquhart, J & Leufkens, H G (1999) Venous thromboembolism among new users of different oral contraceptives *Lancet*, **354**, 127–128

Holm, J , Zoller, B , Svensson, P J , Berntorp, E Erhardt, L & Dahlback, B (1994) Myocardial infarction associated with homozygous resistance to activated protein C *Lancet*, **344**, 952–953

Inbal A Freimark, D , Modan B , Chetrit A , Matetzky, S , Rosenberg, N , Dardik, R , Baron, Z & Seligsohn, U (1999) Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males *Blood*, **93**, 2186–2190

Irani-Hakime, N , Tamim, H , Elias, G Choueiry, S , Kreidy, R , Daccache, J L & Almawi, W Y (2001) Factor V R506Q mutation-Leiden an independent risk factor for venous thrombosis but not coronary artery disease *Journal of Thrombosis and Thrombolysis*, **11**, 111–116

Kontula, K , Ylikorkala, A , Miettinen, H , Vuorio, A , Kauppinen-Makelin, R , Hamalainen, L , Palomaki, H & Kaste, M (1995) Arg506Gln factor V mutation (factor V Leiden) in patients with ischaemic cerebrovascular disease and survivors of myocardial infarction *Thrombosis and Haemostasis*, **73**, 558–560

Mansourati, J , Da Costa, A , Munier, S , Mercier, B , Tardy, B , Ferec, C , Isaaz, K & Blanc, J J (2000) Prevalence of factor V Leiden in patients with myocardial infarction and normal coronary angiography *Thrombosis and Haemostasis*, **83**, 822–825

Martinelli, I , Sacchi, E., Landi, G , Taioli, E , Duca, F & Mannucci, P M. (1998) High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives *New England Journal of Medicine*, **338**, 1793–1797

Martinelli, I , Taioli, E , Bucciarelli, P , Akhavan, S & Mannucci, P M (1999) Interaction between the G20210A mutation of the prothrombin gene and oral contraceptive use in deep vein thrombosis *Arteriosclerosis Thrombosis and Vascular Biology*, **19**, 700–703

Miller, S A , Dykes, D D & Polesky, H F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells *Nucleic Acids Research*, **16**, 1215

Poort, S R , Rosendaal, F.R., Reitsma, P H & Bertina, R M (1996) A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis *Blood*, **88**, 3698–3703

Prohaska, W , Mannebach, H , Schmidt, M , Gleichmann, U & Kleesiek, K (1995) Evidence against heterozygous coagulation factor V 1691 G–> A mutation with resistance to activated protein C being a risk factor for coronary artery disease and myocardial infarction *Journal of Molecular Medicine*, **73**, 521–524

Ridker, P M , Hennekens, C H , Lindpaintner, K , Stampfer, M J , Eisenberg, P R & Miletich, J P (1995a) Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men *New England Journal of Medicine*, **332** 912–917

Ridker, P M , Miletich, J P , Stampfer, M J , Goldhaber, S Z , Lindpaintner, K & Hennekens, C H (1995b) Factor V Leiden and risks of recurrent idiopathic venous thromboembolism *Circulation*, **92**, 2800–2802

Ridker, P M , Miletich, J P , Hennekens, C H & Buring, J E (1997) Ethnic distribution of factor V Leiden in 4047 men and women Implications for venous thromboembolism screening *Journal of American Medical Association*, **277**, 1305–1307

Ridker, P M , Hennekens, C H & Miletich, J P (1999) G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men *Circulation*, **99**, 999–1004

Rosendaal, F R , Siscovick, D S Schwartz, S M , Psaty B M , Raghunathan, T E & Vos, H L (1997a) A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women *Blood*, **90**, 1747–1750

Rosendaal, F R , Siscovick, D S , Schwartz, S M Beverly R K , Psaty, B M , Longstreth Jr W T , Raghunathan, T E , Koepsell T D & Reitsma P H (1997b) Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women *Blood*, **89** 2817–2821

Rosendaal, F R , Doggen, C J Zivelin A , Arruda V R , Aiach M , Siscovick, D S , Hillarp, A Watzke H H , Bernardi, F Cumming, A M , Preston, F E & Reitsma, P H (1998) Geographic distribution of the 20210 G to A prothrombin variant *Thrombosis and Haemostasis*, **79**, 706–708

Sidney, S., Petitti, D.B., Quesenberry, C.P. Jr, Klatsky, A.L., Ziel, H.K. & Wolf, S. (1996) Myocardial infarction in users of low-dose oral contraceptives. *Obstetrics and Gynecology*, **88**, 939–944.

Siscovick, D.S., Schwartz, S.M., Rosendaal, F.R. & Psaty, B.M. (1997) Thrombosis in the young: effect of atherosclerotic risk factors on the risk of myocardial infarction associated with prothrombotic factors. *Thrombosis and Haemostasis*, **78**, 7–12.

Smiles, A.M., Jenny, N.S., Tang, Z., Arnold, A., Cushman, M. & Tracy, R.P. (2002) No association of plasma prothrombin concentration or the G20210A mutation with incident cardiovascular disease: results from the cardiovascular health study. *Thrombosis and Haemostasis*, **87**, 614–621.

Tanis, B.C., van den Bosch, M.A., Kemmeren, J.M., Cats, V.M., Helmerhorst, F.M., Algra, A., Van der Graaf, Y. & Rosendaal, F.R. (2001) Oral contraceptives and the risk of myocardial infarction. *New England Journal of Medicine*, **345**, 1787–1793.

Thomson, D.M., Brown, N.N. & Clague, A.E. (1992) Routine use of hair root or buccal swab specimens for PCR analysis: advantages over using blood. *Clinica Chimica Acta*, **207**, 169–174.

Van Bockxmeer, F.M., Baker, R.I. & Taylor, R.R. (1995) Premature ischaemic heart disease and the gene for coagulation factor V. *Nature Medicine*, **1**, 185.

Van de Water, N.S., French, J.K., Lund, M., Hyde, T.A., White, H.D. & Browett, P.J. (2000) Prevalence of factor V Leiden and prothrombin variant G20210A in patients age < 50 years with no significant stenoses at angiography three to four weeks after myocardial infarction. *Journal of American College of Cardiology*, **36**, 717–722.

Vandenbroucke, J.P., Koster, T., Briet, E., Reitsma, P.H., Bertina, R.M. & Rosendaal, F.R. (1994) Increased risk of venous thrombosis in oral-contraceptive users who are carriers of factor V Leiden mutation. *Lancet*, **344**, 1453–1457.

Vandenbroucke, J.P., Bloemenkamp, K.W., Rosendaal, F.R. & Helmerhorst, F.M. (2000) Incidence of venous thromboembolism in users of combined oral contraceptives: Risk is particularly high with first use of oral contraceptives. *British Medical Journal*, **320**, 57–58.

WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception (1997) Acute myocardial infarction and combined oral contraceptives: results of an international multicentre case-control study. *Lancet*, **349**, 1202–1209.